PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
PCT25863		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IT2005/000088	17.02.2005	25.02.2004

International Patent Classification (IPC) or national classification and IPC
INV. C07K16/18 C07K14/755 G01N33/574 G01N33/58 G01N33/60 C12N15/12

Applicant
UNIVERSITA DEGLI STUDI DI ROMA "TOR VERGATA" et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.12.2005	11.07.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mandl, B Telephone No. +49 89 2399-8434

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IT2005/000088

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-19	as originally filed
Sequence listings part of the description, Pages	
1, 2	as originally filed
Claims, Numbers	
1-26	received on 31.12.2005 with letter of 19.12.2005
Drawings, Sheets	
1/2, 2/2	as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3-10,12-14,16-21,23-26
	No:	Claims	1,2,11,15,22
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: LAKINS J ET AL: "Clusterin biogenesis is altered during apoptosis in the regressing rat ventral prostate." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 43, pages 27887-27895, 23 October 1998

D2: WO 03/086326 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 23 October 2003

D3: WELLMANN A ET AL: "Detection of differentially expressed genes in lymphomas using cDNA arrays: Identification of clusterin as a new diagnostic marker for anaplastic large-cell lymphomas" BLOOD, vol. 96, no. 2, pages 398-404; 15 July 2000

D4: REDONDO M ET AL: "Overexpression of clusterin in human breast carcinoma." AMERICAN JOURNAL OF PATHOLOGY, vol. 157, no. 2, pages 393-399; August 2000

D5: XIE MIN-JUE ET AL: "Expression of clusterin in human pancreatic cancer" PANCREAS, vol. 25, no. 3, pages 234-238; October 2002

D6: SCALTRITI MAURIZIO ET AL: "Clusterin (SGP-2, ApoJ) expression is downregulated in low- and high-grade human prostate cancer." INTERNATIONAL JOURNAL OF CANCER, vol. 108, no. 1, pages 23-30; 1 January 2004,

1. **The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,2,11,15 and 22 is not new in the sense of Article 33(2) PCT for the following reasons:**

i. Document **D1** discloses the production of a panel of monoclonal and polyclonal antibodies each specific for at least one isoform of rat clusterin, including a 42 kDa non-glycosylated isoform which appears to be retained in the nucleus, and a corresponding method for the detection of clusterin levels in biological samples (D1: abstract, table 1, figure 3). One antibody is specific for residues 133-148 of rat clusterin, i.e. amino acid sequence NGDRIDSLLESDRQQSC. 10 consecutive amino acids of this sequence are identical to the corresponding region in the human clusterin (isoforms 1 and 2). Consequently, the antibody of D1 is capable of binding to at least one isoform of human clusterin.
The term "*oligoclonal*" is not considered as representing a specific technical feature that

can be used to distinguish the antibodies of the present application from the monoclonal and polyclonal antibodies of D1 because the term has no specific meaning for the skilled person (see point VIII) and because the production of the antibodies in the examples of the present application do not appear to be different from the production of the polyclonal antibodies of D1. Thus, the disclosures made by D1 anticipate the novelty of claims 1,2 15 and 22.

ii. Document **D2** discloses antigenic epitopes comprising the amino acid sequences SEQ ID NO: 1-4 (D2: Table 1). Thus, the disclosures made by D2 anticipate the novelty of claim 11.

2. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 3-10,12-14,16-21 and 23-26 does not involve an inventive step in the sense of Article 33(3) PCT.

i. The document **D1** is regarded as being the closest prior art to the subject-matter of the present application because it discloses a panel of monoclonal and polyclonal antibodies each specific for at least one isoform of clusterin including glycosylated and non-glycosylated and cytoplasmic and nuclear isoforms.

ii. The antibodies of claims 3, 4 and 12 differ from the antibodies disclosed D1 in that they are specific for different epitopes in the human clusterin molecule.

iii. The problem to be solved by the present invention may therefore be regarded as the provision of alternative antibodies which are specific for different epitopes in the human clusterin molecule.

iv. The solution proposed in claims 3, 4 and 12 of the present application cannot be considered as involving an inventive step because in the absence of any specific and surprising technical effect associated with the epitopes listed in claims 3 and 4 it is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed. Consequently, the subject-matter of claims 3, 4 and 12 is not considered to involve an inventive step.

v. Dependent claims 5-10, 13 and 14 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step because they only relate to features, i.e. various tagging options, which are well known in the antibody field. Thus, claims 5-10, 13 and 14 lack an inventive step.

vi. Claims 16-26 relate to immunological methods for tumour diagnosis and the prediction of the malignancy grade. For this subject-matter document **D1** is regarded as being the closest prior art because it discloses immunological methods for the specific identification of the various isoforms of clusterin including glycosylated and non-glycosylated and cytoplasmic and nuclear isoforms.

vii. The difference between the immunological methods of claims 16-26 and the methods disclosed in D1 is that a specific diagnostic application for tumour detection and/or categorization is referred to.

viii. The problem to be solved by claims 16-26 of the present invention may therefore be regarded as development of diagnostic assays on the basis of antibodies capable of specifically distinguishing between the various isoforms of clusterin.

ix. The solution proposed in claims 16-26 of the present application, i.e. diagnostic applications for tumour detection and/or categorization cannot be considered as involving an inventive step because it was already well known in the art that differential expression of clusterin correlates with tumour progression as it can be seen, for example, from the following documents:

- **D3** demonstrates that clusterin is differentially expressed in lymphoma cell lines and proposes its use as diagnostic marker for anaplastic large-cell lymphoma.
- **D4** reports a significant increase of cytoplasmic clusterin expression during the development from primary to metastatic breast carcinomas and describes clusterin as important factor for determining the aggressive nature of a tumour.
- **D5** points out that the downregulation of clusterin may be involved in the progression of pancreatic cancer.
- **D6** correlates clusterin expression with tumour progression in prostate cancer.

Moreover, D6 specifically proposes to produce antibodies capable of recognizing structurally distinct isoforms of clusterin.

x. Thus, in the light of the prior art, it is considered obvious to develop a method for tumour diagnosis and the prediction of a malignancy grade on the basis of antibodies capable of distinguishing between the various isoforms of clusterin. Consequently, no inventive step can be acknowledged for the subject-matter of claims 16-26.

Re Item VIII

Certain observations on the international application

i. The term "*oligoclonal*" is objected under **Article 6 PCT** because it is no standard term known to the skilled person, thus it renders the claims unclear. Moreover, "*oligoclonal antibodies*" as defined on page 8 of the application appear to require a specific method of making. However, in the example, the method appears to be identical to any other method of making polyclonal antibodies. Consequently, claims relating to "*oligoclonal antibodies*" are not supported as required by **Article 5 PCT**.

ii. A lack of clarity (**Article 6 PCT**) is also observed because there is an inconsistency in the description with regard to antibodies specific for SEQ ID NO:2 because these antibodies are explained to be specific for both the glycosylated and the non-glycosylated isoform of clusterin and, nevertheless, can be used to distinguish between the different isoforms.

iii. Another clarity objection arises from the fact that SEQ ID NO:1 is not 100% identical to the corresponding epitope of any known isoform of clusterin because the amino acid sequence EDQ**K** as present in SEQ ID NO: 1 of the present application does not exist in any naturally occurring clusterin, where the sequence is EDQY. Consequently, is appears possible that the epitope characterized by SEQ ID NO:1 will also give rise to antibodies which are not capable at all of binding to clusterin. SEQ ID NO: 1 can therefore not be used to define an antibody specific for clusterin.

iv. The term "immunogenic" is not considered a technical feature that can be used to distinguish one epitope from another because, when presented in the proper way, any epitope is immunogenic.